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Insights on processes of evolutionary tumor growth

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Abstract

Application of traditional somatic evolutionary theory can offer an appropriate context for studying tumor growth at the molecular level. However, high degrees of heterogeneity (especially genome-level heterogeneity) within tumors coupled with a lack of common driver mutations have posed a challenge to the generally accepted stepwise concept of cancer evolution, where clonal expansion is the key. In order to account for multiple levels of heterogeneity and better understand tumor growth and progression, a new, holistic conceptual framework must be applied in cancer research. Herein, we discuss one such framework, the genome theory of cancer evolution, with respect to tumor growth. This includes detailing the ultimate importance of chromosome aberrations in cancer, the somatic cell evolutionary pattern, and single-cell/population growth dynamics. Under this new framework, tumor growth is a highly dynamic process where emergent outlier subpopulations can greatly influence the pattern of progression and the direction of evolution. Further, genome level changes have a greater impact on cancer evolution than individual gene mutations in most cancer types, as karyotype alteration often results in altered system inheritance which defines the network structure and even can change the meaning of individual genes (representing 'parts inheritance' by changing the gene context. Based on this analysis, we call for a focus shift back on cytogenetic and cytogenomic alterations (especially on non-clonal chromosomal aberrations) in monitoring population growth, identifying the emergence of new subpopulations and studying patterns of evolutionary dynamics. This new insight has implications in understanding cancer evolution in general as well as searching for new diagnostic and treatment strategies.

Introduction

Cancer progression represents an evolutionary process (Gatenby et al., 2009; Greaves and Maley, 2012; Heng et al., 2006a; Merlo et al., 2006; Nowell, 1976). There are heritable variations (genetic, epigenetic, genomic), and the variants display different levels of fitness, which are essential for tumor populations to adapt and grow. While accepted, detailed mechanisms of how somatic cell evolution works are not well understood. These include the relationship between tumor evolution and tumor growth, the emergence of dominant cell populations, and the dynamics of tumor populations under high levels of stress, such as chemotherapy.
To elucidate these mechanisms, efforts are needed to re-examine various types of heritable variations and how these variations contribute to cancer evolution.
The study of classical molecular evolution focuses largely on gradual gene-level change over time. Cancer evolution research has followed the same paradigm, reasoning that individual genetic or epigenetic alterations and the molecular pathways they participate in result in increased fitness, driving the growth of cancer. According to gene mutation

theory, cancer is the result of a stepwise accumulation of small changes in commonly shared genes, so the logical approach is to look for specific gene mutations that drive cancer evolution. Thus, in order to study tumor growth at the genetic level, identification of common genetic aberrations (e.g. universal chromosomes or shared key gene mutations) is key, which in turn would serve as potential diagnostic and therapeutic targets to eradicate cancer.

Unfortunately, this approach has been proven less successful to explain most cancers, aside from exceptional cases including chronic phase chronic myeloid leukemia (Horne et al., 2013a). Further, increased efforts to identify common drivers have resulted in massive amounts of varying and conflicting data. Most solid tumors are marked by high degrees of intra- and inter-tumor genome heterogeneity at multiple genetic and non-genetic levels, and this was recently confirmed with high-throughput sequencing (Gerlinger et al., 2012; Heng et al., 2009; Heppner, 1984). The high degrees of heterogeneity characteristic of tumors coupled with a lack of shared driver mutations have posed a challenge to the stepwise concept of cancer evolution (Heng, 2007a; Heng et al., 2013a; Podlaha et al., 2012). More troubling, this has resulted in confusion in the field, as these results would suggest that individual genes and pathways offer only a minimal contribution to the general cancer patient population and thus hold limited clinical value.

One of the major contributions of cancer genome sequencing is the confirmation of previous cytogenetic findings, which have shown that genome level alteration is key for most cancers. Cytogenetic studies and genome sequencing efforts have revealed high rates of chromosomal abnormalities in clinical samples. Subsets of genome chaos (rapid, stochastic chromosome fragmentation and reorganization) including chromothripsis and chromoplexy have been detected in various types of cancer, and chaotic genomes have been displayed in the majority of cases of certain cancer types (Baca et al., 2013; Heng et al., 2011a; Heng et al., 2011b; Liu et al., 2014; Stephens et al., 2011). These chromosomal aberrations are necessary for the progression of cancer as they provide tumor populations heterogeneity and thus immense evolutionary potential. Chromosomal alterations can drastically impact cells at the genetic and phenotypic levels, including altering a cell's transcriptome and proliferation rate (Abdallah et al., 2013; Kreso et al., 2013; Stevens et al., 2014). Changes of this magnitude explain the relative paltry impact that individual genes and pathways seem to have in the face of genome alteration mediated macro-evolution. Changes in chromosomal topology can have far greater effects on tumor phenotype than changing individual pathways by gene mutation. That is the reason why there are so many different types of non-clonal chromosomal aberrations (NCCAs) detected in various cancers and other diseases (Gisselsson and Hoglund, 2005; Heng et al., 2004; Heng et al., 2013b; Horne et al., 2014a).

To make sense of this heterogeneity and better understand tumor growth and cancer progression, a new conceptual framework must be applied in cancer research that accounts for and unifies the molecular diversity of the disease (Heng et al., 2010a; Horne et al., 2014b; Ye et al., 2009). One such framework is the genome theory of cancer evolution (Heng et al., 2006a; Heng et al., 2006b; Heng, 2009). Herein, we discuss genome-mediated cancer evolution as it pertains to tumor growth. Specifically, we underscore the importance of chromosome aberrations in cancer, review the somatic cell evolutionary pattern and describe single cell and population growth dynamics in this context. Interestingly, under this holistic framework, tumor growth is a highly dynamic process where emergent outlier subpopulations can greatly influence further progression. These new findings are essential to understanding the process of tumor evolution, as growth patterns, drivers, and overall tumor progression rely on multiple levels of heterogeneity. Finally, we call for a re-emphasis on cytogenetic and cytogenomic alterations in monitoring population growth, identifying the emergence of new subpopulations and studying patterns of evolutionary dynamics. In most cancer types, especially solid tumors, genome level changes have a greater impact on cancer evolution compared to individual gene mutations, especially during the punctuated phase where macro-cellular evolution dominates, which differs from the diversification phase (Horne et al., 2014b). This new insight has implications in cancer treatment strategies, as the key to improving patient outcome may lie in tumor constraint rather than aiming to maximize tumor cell death.

Genome theory emphasizes the ultimate importance of chromosome aberrations in cancer

At the center of this new framework is the redefinition of the genome, the highest level of genetic organization. More than the complete genetic sequence, the three-dimensional genome topology defines and governs the overall genetic network. The genome also acts as the main selection platform in evolution. This is evidenced by the constraint applied to genome integrity during sexual reproduction, resulting in the preservation of the karyotype or species identity (Gorelick and Heng, 2011; Heng, 2007b; Horne et al., 2013b; Wilkins and Holliday, 2009). This high level of constraint does

not apply to asexual somatic cell evolution, allowing for rapid cellular evolution to occur through various chromosomal aberrations within individuals.

Under the genome theory, new karyotypes define new system inheritance (as opposed to genes, which define parts inheritance) (Heng et al., 2011a; Heng, 2009; Heng, 2013). This explanation is often easiest to comprehend with the aide of analogy. Imagine that each individual gene is a building material - red brick, lumber, tile, etc. These are necessary to build any kind of building yet, depending on how they are arranged, the final results will be drastically different - a house, a skyscraper, a laboratory. In this analogy, the genome is the blueprint that determines how the various materials (genes and their encoded products) will come together to ultimately form the complete structure (the cell). The three-dimensional architecture of the genome therefore governs the structure of the genetic and protein networks. Simply changing the genomic topology drastically alters the relationship among gene interaction, despite similar gene content. This has been supported by a recent study where karyotypic alterations were shown to influence gene expression profiles, and by single cell sequencing of glioma (Patel et al., 2014; Stevens et al., 2014). In addition, evidence from yeast studies strongly supports that aneuploidy directly affects gene expression and results in phenotypic variation (Pavelka et al., 2010). Genome heterogeneity has recently been linked to growth heterogeneity (Abdallah et al., 2013), further supporting the relationship between karyotype and phenotype. Genome-level alteration therefore results in new system generation defined by new system inheritance. This holds critical importance in tumor growth and progression, as karyotypic change can potentially result in formation of an aggressive phenotype. This would then contribute to further tumor progression. Thus from an evolutionary standpoint, the importance of stochastic genome aberrations in cancer is to increase the evolutionary potential of the disease through increased genome system heterogeneity, which generates a wide array of phenotypes and maximizes the odds of survival upon selection.

In the past, most genetic studies were performed using model systems displaying stable genomes (e.g. green pea, fly, corn, lab mice). As a result, any system inheritance (blueprint) contributions would be subtracted (invisible), allowing for illustration of a close genotype (mainly the gene level) and phenotype relationship. Cancer genetic studies, in contrast, are completely different. Cancer evolution is driven by genome replacement, making system inheritance the most important inheritance while making gene-mediated parts inheritance become trivial. Interestingly, in cancer, the mechanism(s) maintaining various types of inheritance become less precise, forming a new type of inheritance, called fuzzy inheritance, which provides the mechanism to explain the high level of heterogeneity in cancer (Heng 2015; Heng et al., 2014; Abdallah et al., unpublished observations).

Without the above understanding, and perhaps also due to the overemphasis on gene-based research, many key facts about chromosomal aberrations in cancer are not well known by most molecular cancer researchers. These include the fact that chromosomal change is necessary to induce all key transitions during cancer evolution, including cellular transformation. Even in studies which claim to demonstrate the effect of specific genes in tumorigenesis, chromosomal alterations are often observed in those cells driven to become cancerous (Elenbaas et al., 2001; Heng, 2007a; Heng et al., 2010a; Li et al., 2000). This explains why in cancer gene-specific knockout animals, different karyotypes and molecular pathways are found to be present in different animals' tumors (Bassing et al., 2003; Heng et al., 2006a; Sharpless et al., 2001). Additionally, higher levels of NCCAs have been closely linked to more aggressive tumors, solidifying the idea that chromosomal change is necessary for cancer to evolve and grow (Galipeau et al., 2007; Ye et al., 2009;). Tumors with high levels of chromosome heterogeneity have also been linked to lower patient survival rates, further highlighting the clinical importance of this phenomenon (Hicks et al., 2006; Holland and Cleveland, 2012).

The concept that only highly penetrant, specific genetic alterations are important for cancer progression has contributed to the lack of appreciation of the importance of stochastic chromosomal aberrations as well. The chromosome changes mentioned above differ from well-known clonal chromosomal aberrations (CCAs), such as the Philadelphia chromosome detected in chronic myeloid leukemia. These changes do not occur in any particular pattern with high frequency, but rather they are stochastic, and the prevalence of any particular karyotype in a tumor is due to the competitive advantage it confers. The seeming randomness of karyotypic change in solid tumors fits well within the punctuated cancer evolution framework. Furthermore, the formation of unique genomes in cancer is incredibly common; there is proclivity for chromosomal change during every cell division when the genome is highly unstable.

The tradition of ignoring the non-specific changes in the cancer research field is the reason that these stochastic chromosomal aberrations have only recently been reconsidered as drivers of tumorigenesis and tumor growth (Castro-Gamero et al., 2013; Heng et al., 2004; Heng et al., 2006a; Heng et al., 2006b; Heng et al., 2006c; Heng et al., 2010a; Klein et al., 2010; McCormack et al., 2013; Podlaha et al., 2012; Stepanenko et al., 2013; Stepanenko and Kavsan, 2013; Valind and Gisselsson, 2014). The

crucial realizations that have led to this reconsideration are: chromosome alterations change genome-defined systems; the high level of NCCAs is essential for cancer evolution (Heng et al., 2006a; Heng et al., 2013a); and massive and seemingly stochastic chromosomal alterations seem to be the only shared findings among many cancer types. Knowing the genome variation plays the important role in cancer evolution, we should not continue the practice of focusing solely on CCAs.

The somatic cell evolutionary pattern

The two phases of cancer evolution were originally based on the karyotype pattern observed in an immortalization model where both non-clonal and clonal expansions were detected (Heng et al., 2006a), and have since been confirmed in breast cancer using single-cell genome sequencing (Navin et al., 2011; Wang et al., 2014).

Cancer evolution is a series of genome-mediated system replacements occurring in dynamic cycles of NCCAs and CCAs occurring within the two evolutionary phases (Figure 1).

In the stepwise phase, the majority of cells are clonal across generations, and karyotypic diversification is traceable.

The punctuated phase is characterized with a high frequency of NCCAs and massive genome reorganization, which break multiple system constraints (e.g. genome integrity, tissue architecture, etc.).

Thus, cancer progression consists of both macro-cellular (genome system replacement) and micro-cellular (modification of the genome-defined system) evolution. There is an increased support for the concept of macro-micro phases of evolution in cancer (Klein, 2013).

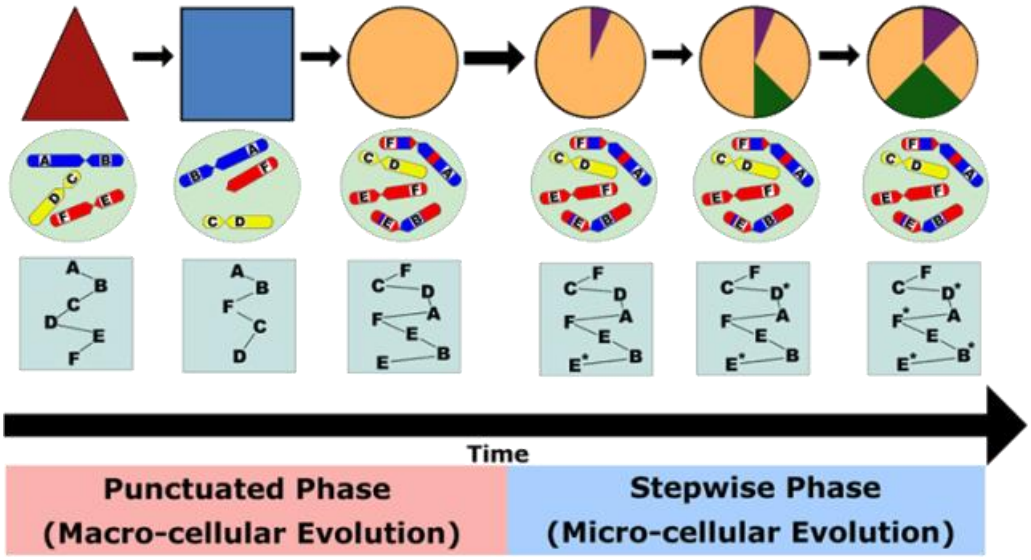

Figure 1. Stochastic model of genome-mediated cancer evolution. Cancer evolution is divided into two distinct evolutionary phases, the punctuated stochastic phase (or macro-evolutionary phase) and the stepwise gradual phase (or micro-evolutionary phase). Punctuated phases are marked by extreme heterogeneity and rapid genome changes, represented by genome system changes over time, with each shape representing a unique genome system. Different chromosomes are designated by color (red, yellow, blue) and drawn within the nucleus below the corresponding system. Genes are designated A, B, C, D, E, F within the chromosomes, and corresponding protein networks are illustrated below by the relationships between proteins A, B, C, D, E, F. The punctuated phase is caused by system instability-mediated macro-cellular evolution, resulting in high NCCA frequency illustrated by different genome systems (shapes), topologies (karyotypes, including numerical and/or structural chromosome aberrations), and protein interactive networks. Following selection pressure, a unique genome system survives (circle). In contrast to genomes in the punctuated phase, this genome system in the stepwise phase remains relatively stable over time, although it does acquire low-level changes (represented by pie piece changes) such as gene mutations, epigenetic alterations and/or small traceable genome-level alterations that aide in adaptation. Genetic/epigenetic alteration is indicated by asterisks (*) in the protein network. These micro-cellular changes can be classified into clonal expansion and diversification. Thus, the stepwise phase is mainly associated with system stability and micro-cellular evolution. Only one run of the NCCA/CCA cycle is presented.

Knowing the two phases of cancer evolution also helps in understanding cellular evolutionary convergence and divergence of cell populations. In addition to distinguishing the evolutionary changes that can be traced at genome or gene levels, the punctuated phase represents a window where drastic divergence frequently occurs. Interestingly, phenotype convergence (such as immortalization,

metastasis and drug resistance) is often associated with karyotype divergence. In contrast, within the stepwise phase, genotype and phenotype convergence might be observed.

The evolutionary mechanism of cancer takes into account that every factor (genetic, non-genetic, internal, external) can contribute to cancer evolution as long as it functions as a source of stress to the system, particularly when it triggers genome instability (Heng et al., 2013a). The evolutionary mechanism of cancer is equal to the sum of all individual molecular mechanisms (Heng et al., 2010a) and can be described in three steps: 1) stress-induced genome system instability; 2) resulting heterogeneity at multiple levels (e.g. genomic, genetic, epigenetic); and 3) somatic cell evolution. The most effective way to drastically increase evolutionary potential and drive disease progression is through genome-level alteration, which yields new genome systems rather than network adjustments that may result from gene-level change. The evolutionary mechanism of cancer can also be explained by multiple level landscape models (Heng et al., 2013a; Horne et al., 2014b; Huang, 2013).

These concepts explain the complexity of cancer, including the highly dynamic profiles displayed in inter- and intra-tumor samples, the trade-off between short-term adaptation and the potential price to pay in the long term (Horne et al., 2014a), and the unpredictable responses for the majority of chemotherapeutic, or even target-specific, drug interventions. As mentioned, NCCAs pay a crucial role in disease progression by increasing the evolutionary potential. Induction of genome chaos has been associated with a wide variety of stresses, including chemotherapeutics (Liu et al., 2014). Thus, as the administration of high-dose chemotherapeutics can be initially effective in reducing tumor cell numbers, it can also result in the generation of new NCCAs, ultimately giving the disease a fighting chance for recovery and resistance by increasing the evolutionary potential and generating aggressive outlier subgroups.

Individual cell and population growth

This new framework provides new perspective to tumor growth at the population and single cell levels. Macro- and micro-cellular evolution can now be reflected by the dynamic relationship between NCCAs and CCAs. This means that cancer progression is not only a process of gradual genetic tinkering and selection (reflected by CCAs) as once thought. Macro-cellular evolution involves drastic genome system reorganization, meaning that tumor cells can rapidly generate a wide range of growth profiles to be selected upon during the punctuated phase (reflected by low rates of NCCAs in the stable phase and extremely high rates during the punctuated phase). This new framework also suggests that tumor population growth is a far more dynamic process, where aggressive populations can emerge and drive growth, but also can be overcome by other subgroups further in progression. This is very different from traditional thinking, where a single, traceable lineage is believed to drive overall growth and progression throughout the duration of the disease.

NCCAs provide karyotypic differences, which affect many phenotypic traits of cells. In the context of tumor growth, the effect of NCCAs on the growth rate of cells is especially important. NCCAs and CCAs represent survival and growth advantages respectively (Ye et al., 2007). Cells grown from single clones in populations with unstable karyotypes have wildly more variance in growth rates when compared with karyotypically stable cell lines - and even these 'stable cell lines have more variance than would be expected if each daughter cell was a perfect copy of its mother (Abdallah et al., 2013; Kreso et al., 2013). Thus, unstable cancer cells produce populations of cells consisting of individuals growing at drastically different rates.

Therefore, the outliers rule in cancer evolution, not the average cells in the population. The potential for fast-growing outlier cells to drive tumor growth has been demonstrated in clonal cells. The population doubling time of cells from the same subpopulation has been shown to fluctuate, and subpopulations of cells with unstable genomes have been observed to grow at much higher rates (Abdallah et al., 2013). Even a single cell can give rise to heterogeneous growth patterns. In essence, heterogeneity is heritable - an unstable cell in a population is unstable, and thus gives rise to more unstable cells, this leads to a hugely diverse population (Kreso et al., 2013; Abdallah et al., unpublished observations). Fuzzy inheritance aptly explains heterogeneity maintenance. Even in comparatively stable cancer cell lines, the punctuated phase of evolution and phenomena like genome chaos ensure genotypic diversity in the population (Abdallah et al., 2013; Baca et al., 2013; Liu et al., 2014; Meyerson and Pellman, 2011).

The framework also reveals the impact and power of the single cell in tumor growth.

Table 1. Examples of Karyotypic Abnormalities

Category	Type	References
Structural	Translocation	ISCN, 2013
	Deletion	
	Insertion	
	Invertion	
	Duplication	
	Triplication	
	Quadruplications	
	Ring Chromosomes	
	Fission	
	Fragile Sites	
	Dicentric Chromosomes	
	Derivative Chromosomes	
	Telomeric Associations	
	Premature Chromosome Condensation (or Pulverization)	Johnson and Rao, 1970
	Micronuclei	Fenech et al., 2011
	Multipolar mitosis	Gisselsson, 2001
	Chromosome bridge	Gisselsson, 2001
Numerical	Aneuploidy	ISCN, 2013
	Polyploidy	
	Endopolyploidy	
Non-Traditional/ Newly Identified	Free Chromatin	Heng et al., 1988a; Heng et al., 1992; Heng et al., 2013b
	Defective Mitotic Figures	Heng et al., 1988a; Heng et al., 2004; Haaf and Schmid, 1989; Smith et al, 2001
	Sticky Chromosomes	Heng et al., 2013b
	Unit Fibers	Bak et al., 1979; Heng et al., 1988b
	Chromosome Fragmentation (C-Frag)	Stevens et al., 2007
	Genome Chaos/Karyotype Chaos	Heng et al., 2006a; Heng 2007c; Duesberg, 2007; Liu et al., 2014
	Karyoplast Budding	Walen, 2005
Unclassified	Nuclei with small holes	Heng et al., 2013b
	Giant nuclei	Heng et al., 2013b

Recently, single-cell and population based experiments have shown that, within a heterogeneous population, aggressive outliers influence the growth of the entire population through rapid increase of cell numbers (Abdallah et al., 2013). Taking macro-cellular evolution into consideration, aggressive outlier cells are rapidly generated and drive tumor growth. Thus, the population dynamics of the tumor are influenced by rapid formation and transient contributions of outlier groups. However, current average-based technologies do not account for these outliers, as these data are washed away in these types of analyses. In fact for unstable cell populations, the average is irrelevant, as it does not exist within the population (Abdallah et al., 2013)! As a result of averaging, the true contribution of outlier subgroups is lost, and our understanding of tumor growth is skewed. In order to appropriately gauge the patient's disease status (genome heterogeneity and stability), application of single-cell based techniques (e.g. spectral karyotyping, single-cell sequencing) is essential before and during treatment regimens.

Future perspective

The above analyses illustrate the ultimate importance of cytogenetic and cytogenomic approaches in cancer research, especially for studying cell population behavior and the pattern of macro-cellular evolution. In the future, monitoring karyotype change will be essential for gene-based cancer research, as karyotype alteration likely results in the formation of new molecular network structures, which also change the gene context. In addition, as cancer is a highly dynamic evolutionary process, and no simple constant clonal expansion pattern driven by gene mutation accumulation is shared across all studies (especially in drug resistance studies), cytogenetic monitoring must be applied to define the context. Furthermore, cytogenetic approaches can provide low cost individual cell and cell population profiling.

In order to better understand the dynamic growth of cancer cells and the evolutionary pattern, we call for a shift from continuing collecting lower level molecular data that offer little clinical value towards monitoring the system behavior of cancer in an

evolutionary, holistic context. Achieving this requires focus at the genome level, and importantly, inclusion of NCCAs in analyses. This includes both understanding the various types of chromosomal aberrations (Table 1) as well as incorporating NCCA frequencies in measurements of genome instability.

First, there are still many unclassified and/or yet to be generally accepted chromosomal aberrations frequently observed from cytogenetic preparations of cancer materials that need to be characterized or scored (Heng et al., 1988a; Heng et al., 2004; Heng et al., 2013). According to our analyses, these chromosomal aberrations represent evolutionary potential and provide reliable biomarkers of the entire tumor cell population.

Second, despite the importance of chromosomal instability (CIN) studies in cancer, traditional cytogenetic analysis has focused on CCAs, and NCCAs have been dismissed as insignificant genetic "noise". To change this situation, it is necessary to use frequencies of NCCAs rather than CCAs to measure CIN, and to study and classify diverse types of NCCAs and compare the relationships among these chromosomal aberrations. NCCAs provide diversity to the population, as a population consisting of many NCCAs is more robust, and thus has a greater amount of evolutionary potential (Heng et al., 2013a; Liu et al., 2014). The fact that NCCAs are so common suggests that such a potential is required for most cancer populations to survive and grow. The status of NCCAs can also be used to differentiate tumor types, distinguish cancer stages, and predict treatment response (Duesberg et al., 2007; Heng 2007a; Heng et al., 2010b; Ye et al., 2009).

Accounting for tumor cell growth heterogeneity is also crucial, given recent findings regarding the impact of outlier influence in cancer population growth. Like cancer genome analyses, cancer growth analyses require a shift away from averaging methods, as these wash away outlier contributions and provide a misrepresentation of the population (Abdallah et al., 2013). In addition, further work is needed in understanding the growth pattern differences between NCCAs and CCAs. The heterogeneous growth (and death) of tumor cells means that many cancer cells will die during progression (Stevens et al., 2013), but the overall tumor population marches on, and this understanding has relevance in treatment regimens and drug resistance. Thus, cytogenetic analysis has the capability to identify and offer prediction power of outliers within unstable cell populations, which can drive the direction of somatic cellular evolution, and this drastically differs from stable cell populations where the "average profile" rules.

Considering the roles of massive genome reorganization (i.e. genome chaos), resultant NCCAs, and aggressive outliers in tumor growth, current therapeutic approaches involving the administration of maximum tolerated doses must be reconsidered. High dose treatment can significantly reduce tumor cell populations initially. However, since varying sources of stress have been previously associated with genome chaos, rapid genome reorganization can be induced, potentially resulting in the generation of aggressive outlier groups. This would then result in the rapid repopulation of the tumor cell population and further drive disease progression and drug resistance. This mechanism can explain some early, promising successes in alternative approaches including adaptive and metronomic therapies. The aim of adaptive therapy is to maintain a stable tumor burden rather than elimination, and this is achieved by therapeutic dose adjustments (Gatenby et al., 2009; Silva et al., 2012). Metronomic therapy aims to eliminate tumors, however, this differs from the maximum tolerated dose strategy as lower drug concentrations are administered in a rhythmic regimen (Kerbel and Kamen, 2004). These approaches both utilize lower doses of chemotherapeutics, which may not induce genome chaos and macro-cellular evolution. Perhaps the key to successfully constraining tumor cell growth and improving patient outcome is through applying genome constraint with milder dosage. In addition, considering that genes are considered moving targets in genome-mediated evolution and various stresses can induce chromosomal instability and genome chaos, emphasis should be placed on determining the appropriate degree of stress when designing regimens rather than the specific target(s). We recently studied the transition cells undergo after treatment. Initially, high cell death is induced. However, cells in culture models recover over time. We have found that harsh initial treatments result in the production of outliers that can outgrow untreated cell populations after recovery (Horne et al., unpublished observations).

Focusing on the overall heterogeneity of the cell population, as reflected by the NCCA frequency and outlier profiles (e.g. chaotic genome complexity), will result in an improved understanding of cancer progression and provide accurate prediction measures. Experimental efforts are urgently needed in order to translate these concepts to clinical application. Clearly, more attention is needed on genome level alteration and genome mediated somatic cell evolution, which play important roles in understanding and predicting the behavior of cell populations and how they grow (Abdallah et al., 2013; Heng et al., 2006a; Horne and Heng, 2014; Ye et al., 2007).

Finally, observing the dynamics of tumor cell growth (both in vivo and in vitro) has provided a unique opportunity to watch somatic cell evolution in action. Since both macro- and micro-cellular

evolution can be observed, tumor cell growth represents a great model to study how the different types of inheritance including gene-mediated parts inheritance, genome-mediated system inheritance, and tissue-specific fuzzy inheritance impact on evolutionary dynamics. Understanding the pattern of tumor cell evolution will lead to new strategies for managing cancer.

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